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Analysis of five alkaloids using surfactant-coated multi-walled carbon nanotubes as the pseudostationary phase in nonaqueous capillary electrophoresis

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ABSTRACT

In this paper, surfactant-coated multi-walled carbon nanotubes (SC-MWNTs) have been proposed as a novel pseudostationary phase (PSP) to enhance the separation of isoquinoline alkaloids in nonaqueous capillary electrophoresis (NACE). Several parameters affecting NACE separation were studied including the MWNT concentration, the electrolyte concentration, pH* and the separation voltage. In comparison to conventional NACE, the addition of an MWNT dispersion using surfactant solutions in the electrolyte produced an important enhancement in the resolution due to the π–π interactions between the analytes and the surface of the carbon nanotubes. Using SC-MWNTs (6 μg mL^{-1}) as a PSP in the background electrolyte (BGE) (i.e., 20 mM sodium acetate in methanol–acetonitrile (80:20, v/v)) provided the complete separation of five alkaloids. Finally, the developed method has been successfully applied to the detection and quantification of the tested compounds of *Rhizoma Coptidis*.

1. Introduction

Capillary electrophoresis (CE) is now a widely applied separation technique that has been successfully used for the analysis of complex compounds due to its high resolution, minimal sample volume requirement, shorter analysis time and high separation efficiency than that of conventional methods [1]. In recent years, pseudostationary phases (PSPs), such as microemulsions [2,3], micelles [4], ionic liquid [5], silica particles [6], molecularly imprinted polymers [7], nanoparticles [8,9], and vesicles [10] have been used to enhance the separation efficiency of investigated analytes and to resolve a variety of different types of compounds with various different solute properties according to their interaction with the electrophoretic system.

Since the discovery of carbon nanotubes (CNTs) [11], these tube-like materials, have attracted considerable attention due to their unique structural, mechanical, and electronic properties [12]. However, their practical uses have been limited due to the inherent insolubility of CNTs in either water or organic solvents resulting from a tendency to form agglomerates or bundles in solvents, which is caused by van der Waals interactions. Recently, noncovalent bonding or physical adsorption have been used to exfoliate the bundles and stabilize individual tubes [13], leading to significant application of functionalized CNTs in CE fields, e.g., as an additive [14], a PSP [15], a sorbent material [16], and an analytical target [17], and in chiral selectors [18,19] in the study of an immobilized stationary phase [20]. Among these investigations, carboxylic single-walled CNTs (C-SWNTs) and carboxylic multi-walled CNTs (C-MWNTs) have been used as additives or PSPs in the background electrolyte (BGE) to improve the separation of caffeine, theobromine, purine, and pyrimidine bases [13,21–23]. In addition, the use of surfactant-coated CNTs or C_{60} in aqueous running buffers was more effective for increasing the resolution and the selectivity of amphenicols, flavonoids, phenolic

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acids, lactams antibiotics, glycosides, and saponins, as well as the enantioseparation of ephedrines and clenbuterol [24,25]. Sodium dodecylbenzenesulfonate, sodium cholate, DAPS, Brij-35, Triton X-100, 1-butyl-3-methylimidazolium tetrafluoroborate (bminBF$_4$), and 1-dodecyl-3-methylimidazolium chloride (C_{12}minCl) have been demonstrated to be the most efficient surfactant-coated CNTs in aqueous running buffers that enhanced the separation efficiency of bioactive compounds in complex preparations [26,27]. However, the CNTs are always applied as a PSP or an additive to the aqueous running buffer.

In comparison to conventional CE, nonaqueous capillary electrophoresis (NACE) [28], which is based on the use of electrolyte solutions prepared from pure organic solvents, has received a remarkable amount of attention from researchers in a number of fields due to its unique properties, such as a high separation selectivity [29], a shorter analysis time, and a low reagent consumption. Whereas the great benefits of NACE are in the separation of analytes that are hydrophobic species and are unstable in aqueous solutions, the solubility of many analytes and additives or selectors is enhanced in organic solvents. In particular, hydrogen-bonding and dipole-related and ionic interactions can be exploited in NACE in a hydrophobic environment, because these hydrophilic interactions are thermodynamically strengthened in nonaqueous media relative to aqueous media [30]. Furthermore, organic solvents offer attractive features for detection systems such as electrospray ionization-mass spectrometry and electrochemical methods, providing improved sensitivity and a wider scope of application [31]. Now, NACE has been successfully applied for the analysis of drugs and pharmaceuticals [32,33], organic environmental pollutants [34,35], plant extracts [36,37], food [38,39], and biological samples [30,40]. To the best of our knowledge, the use of surfactant coated multi-walled CNTs (SC-MWNTs) as a PSP in the NACE system has not been previously reported.

Rhizoma Coptidis (Huanglian in Chinese) is among the more commonly used as herbal drugs in traditional Chinese medicine (TCM). The alkaloids components in *Rhizoma Coptidis* are active constituents and exhibit a great varieties of biological and pharmacological activities including anti-diarrhea, anti-diabetes, anti-microbe, anti-inflammatory and anti-cancer as well as having the ability to dispel dampness, cure toxicosis and detoxify [41–46]. Among alkaloids, berberine (BER) is the major one, approximately 50%, while coptisin (COP), epiberbeine (EPI), palmatine (PAL) and jatrorrhizine (JAT) are the most abundant minor alkaloids. Therefore, concentrations of total alkaloids and individual components are important parameters in evaluating the biochemical activities or marketing the quality of the dose in medical usages [47]. The aim of the current work was to characterize a novel PSP using SC-MWNTs to improve the electrophoretic resolution of five alkaloids of *Rhizoma Coptidis* in NACE. By investigating the effects of the MWNT concentration, the electrolyte concentration, the separation voltage, and the apparent pH (pH*), the CNTs functionalized with a surfactant exhibited a distinct separation mechanism compared to that of conventional NACE and provided satisfactory assay results. The presence of SC-MWNTs in the BGE resulted in good electrophoretic resolution and peak shape due to the surface area and the adsorption capacity of CNTs. Then, we used the method developed to study compounds selected from a plant preparation.

2. Experimental

2.1. Chemicals and reagents

MWNTs with outer diameters (O.D.s) of 10–20 nm and lengths of 5–15 μm were provided by Shenzhen Nanotech Port Co. Ltd., (Shenzhen, China) and used without further purification. Methanol and aceto nitrile were of chromatographic grade and were purchased from the Beijing Chemical Reagent Factory (Beijing, China). Sodium acetate and acetic acid were of analytical-reagent grade and were purchased from Tianjin Chemical Reagent Factory (Tianjin, China), these were used without further purification. Sodium dodecyl sulfate (SDS) was purchased from Sigma–Aldrich Shanghai Trading Co. (Shanghai, China). Berberine, palmatine, coptisine, and epiberberine were provided by the Ronghe Pharmaceutical Technology Development Co. Ltd. (Shanghai, China). Jatrorrhizine was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Fig. 1 shows the chemical structures of the solutes tested. The *Rhizoma Coptidis* drug samples were purchased from a local drugstore (Beijing, China) and were identified as *Coptis. chinensis Franch*.

2.2. Instrumentation and electrophoretic conditions

All of the CE separations were performed on a P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array UV detector (wavelength range from 190 to 600 nm). For data storage and evaluation and CE instrument control, the Karat software (Beckman Instruments) and a personal computer were used. Scanning electron microscopy (SEM) images were obtained with a thermal field emission SEM (SU70, Hitachi, Japan). A pH meter (pH S-3C, Shanghai Precision & Scientific Instrument Co., Ltd., China) equipped with a glass electrode was used to measure the pH* values of the CE running buffers in NACE. The electrode was calibrated using standard aqueous buffers with pH values of 4.00, 6.86 and 9.18 at 25 ± 0.2 °C. Fused silica capillaries were obtained from the Yongnian Optical Fiber Factory (Hebei Province, China). These capillaries had dimensions of 50 μm inner diameter (I.D.) × 365 μm O.D. with an effective length of 32.8 cm and a total length of 50.2 cm. The samples were hydrodynamically injected under 50 mbar of pressure for 5 s. The applied voltage used for the electrophoretic separation was 25 kV at a constant temperature of 25 °C.

Prior to the first use, the new capillaries were conditioned by rinsing with 1 M sodium hydroxide for 20 min, 0.1 M sodium hydroxide for 20 min, deionized water for 10 min, and BGE for 10 min. Between runs, the capillary was rinsed with BGE for 5 min. To ensure the repeatability of the migration time, the separation buffer was replenished after three injections. At the end of the day, the capillary was rinsed for 10 min with 0.1 M sodium hydroxide and for 5 min with water.

The BGE employed for separation was prepared using a (20:80, v/v) methanol–acetonitrile mixture containing 20 mM sodium acetate solution (the pH was adjusted using acetic acid or ammonium hydroxide) with surfactant-dispersed MWNTs. The sample standard solutions were diluted with methanol to obtain a final concentration of 100 μg mL^{-1}.

2.3. Preparation of the CNTs dispersions

The best procedure found to disperse/solubilize the carbon nanotubes consists of introducing 1 mg of the MWNTs into a glass beaker containing 25 mL of a 17.5 mM SDS in methanol and submerging the mixture in an ultrasonic bath for 20 min. The suspension obtained was directly introduced into the electrophoretic capillary for its analysis [48,49].

2.4. Sample preparation

A 0.2 g portion of the *R. coptidis* dried drug powder was accurately weighed and extracted with 50 mL of methanol/HCl (100:1)